Morphological, anatomical and palynological studies of the genus *Zoegea* L. (Asteraceae) in Iran

Khadije Mahmoodi, Maneezheh Pakravan & Valiollah Mozaffarian

Received: 17.05.2017 / Revised: 27.05.2017 / Accepted: 31.05.2017 / Published: 21.12.2018

1Faculty of Biological Science, Alzahra University, Sheikh-Bahae Sq., Tehran, Iran
2Department of Botany, Research Institute of Forests and Rangelands, Agricultural Research Education and Extension Organization (AREEEO), P. O. Box 13185-116, Tehran, Iran

*Correspondent author: pakravan@alzahra.ac.ir

Abstract. The genus *Zoegea* L., belongs to Asteraceae family and has about 10 species in the world. This genus is considered to be an Irano-Turanian and Mediterranean element and is distributed in south-western and central Asia and in the central, southern, north-western and south-western parts of Iran as well. The subspecies classification of the genus is not consensus and various classifications could be found in different taxonomy resources. In this study various specimens from different regions of Iran were studied. In addition, anatomical and palynological characters were used to perform a cluster analysis in order to determine species groups. In the end, our results confirmed that *Z. baldschuanica* and *Z. glabricaulis* were distinct species.

Keywords. anatomy, compositeae, middle east, morphology, palynology, SEM

INTRODUCTION

The genus *Zoegea* L., also known as Khorshid-e Sobh in Persian, belongs to the Asteraceae family and is classified in Cardueae tribe and Centaureinae subtribe. In the Centaureinae subtribe, the high variation of morphological characters makes the taxonomy of the genus highly problematic. *Zoegea* has unusual combination of plesiomorphic morphological characteristics and apomorphic pollen types (Wgenitz & Hellwig, 1996). Involutional bracts characters and basic chromosome number (x=14 and x=15) are plesiomorph. Therefore, it was regarded as an isolated genus in Centaureinae.
Later palynological studies showed that Zoegea has serratula type pollen (Martin & Gacia-Garcia-Jacea 2000). Therefore, both morphological and palynological characteristics had confirmed that Zoegea has a basic status in phylogenetic tree. Based on different phylogenetic studies (Gacia-Jacea et al., 2002; Gacia-Jacea et al., 2001) Zoegea is considered to be a monophyletic genus, but there isn’t a consensus idea on the situation of Zoegea in the subtribe Centaureinae (Funk et al., 2005).

Three species of Zoegea grow in Iran, Turkey, and Egypt and generally in the central and western zones of Asia (Funk et al., 2005; Kubitzki, 2007; Mabberley, 2008). There are 7 taxa in the area of Flora Iranica: Z. purpurea Fres., Zerinita subsp. crinita Boiss., Z. crinita subsp. baldschuanica (C-Winkl.) Rech.f., Z. crinita subsp. glabricaulis (Czer.) Rech.f., Z. leptaurea L. subsp. leptaurea, Z. leptaurea subsp. mesopotamica (Czer.) Rech.f., Z. leptaurea subsp. mianensis (Boiss.) Rech.f. All of these taxa, except Z. leptaurea subsp. leptaurea, were also reported from Iran, although its presence in the whole area of Flora Iranica is doubtful (Wagenitz, 1980). In this treatment four species were reduced to subspecies rank.

Except some palynological studies (Wagenitz, 1955; Wagenitz & Hellwig, 1996; Garcia-Jacas et al., 2002) there are no significant anatomical and morphological studies in the genus Zoegea. In this study, various anatomical and morphological features of Iranian members of the genus Zoegea were investigated for the first time.

**MATERIALS AND METHODS**

**Taxonomy and morphology**

In addition to our own collections (ALUH) from different provinces of Iran (i.e., Fars, East Azarbayjan, Lorestan, Khuzestan and Bushehr), specimens of TARI, TUH, KAR and RNAK herbaria were studied. The specimens that have been used in palynological and anatomical studies are listed in Table 1. In this study, 21 qualitative and 17 quantitative characters of more than 115 plant samples were measured and used for the morphological studies (Table 2). SPSS software (ver. 18) and Ward’s and Canonical Variate Analysis (CVA) methods were used for statistical analysis.

**Table 1.** The list of specimens used in palynological and anatomical studies.

<table>
<thead>
<tr>
<th>Species</th>
<th>Examined specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoegea crinita subsp. baldschuanica</td>
<td>Khorassan province: Sarakhs, Cheshmeshuran, 490 m a.s.l., 21 May 1972, H. Foroughi 4266 (TARI); Kermanshah province: Bistun, Kamijeh, 1600 m a.s.l., 3 June 1997, M. Nemati &amp; A. Qaderi 6599 (RANK).</td>
</tr>
<tr>
<td>Z. crinita subsp. crinita</td>
<td>Hormozgan province: Ca. 170 km from Bandar-Abbas to Sirjan Hajiyabad, 900 m a.s.l., 5 January 1995, V. Mozaffarian 74249 (TARI); Kermanshah province: Paveh, the hill above sarab houli, 1500-1800 m a.s.l., 18 June 1987, Hamzehee 1232 (TARI); Kermanshah province: Bistun mountain, 1340 m a.s.l., 3 June 1997, Nemati &amp; Qaderi 5528 (RANK); Fars province: Bamu protected region, Tange Chah Mahaki, 1800-2000 m a.s.l., 1 June 1975, P. Wendelbo &amp; H. Foroughi 17731 (TARI).</td>
</tr>
<tr>
<td>Z. crinita subsp. glabricaulis</td>
<td>Fars province: Near Khonj, 15 April 1983, M. Assadi &amp; Bazgosha 41581 (TARI); Fars province: 36 km from Khonj to Lar, 700 m a.s.l., 15 April 1983, M. Assadi &amp; Bazgosha 41669 (TARI); Kermanshah province: Qasr-e shirin, Imam Hasan, 600 m a.s.l., 1 June 1996, M. Nemati 6394 (RANK).</td>
</tr>
<tr>
<td>Z. leptaurea subsp. mianensis</td>
<td>Khuzestan province: Sarsadish to Bebbahan, 20 km to bebahan, 700 m a.s.l., F. Attar 11004 (ALUH); Kordestan province: 101 km from Marivan on road to Paveh between Nowsad and Paveh, 1000 m a.s.l., 5 May 1978, H. Runemark &amp; V. Mozaffarian 27435 (TARI); Lorestan province: Khoramabad, 33°42'51&quot;N, 48°26'30&quot;E, 1676 m a.s.l., 31 March 2013, Kh. Mahmoodi 11002 (ALUH).</td>
</tr>
<tr>
<td>Z. purpurea</td>
<td>Hormozgan province: 15 km from diviation of Minab, Rudan road to Rudan, 500 m a.s.l., 5 June 1982, V. Mozaffarian, Banhahsemi &amp; Shahnazadeh 39470 (TARI); Hormozgan province: West slope of Kuh-e Genu, N. of Tazian, 500-900 m a.s.l., 24 April 1985, V. Mozaffarian 49549 (TARI); Esfahan province: Ghameshoo protected area; Koobe Arre Khar, 2200 m a.s.l., 6 June 1996, M. Usoofi 14551 (TARI); Khorassan province: Esferrayan, Shah Jahan Ms. Region Rocky, soily Mt., Tourkan from deep gorge close to Noushivran village, 1400-2500 m a.s.l., 8 June 1984, V. Mozaffarian 48569 (TARI); Yazd province: 14 km to Anarak on the road, from Chupanan, 1450 m a.s.l., 6 June 1986, M. Assadi &amp; Bazgosha 56545 (TARI).</td>
</tr>
</tbody>
</table>
**Table 2. Characters used in morphological study**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Qualitative characteristics</th>
<th>Symbol</th>
<th>Quantitative characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>IT</td>
<td>Indumentums Type</td>
<td>LH</td>
<td>Herb Length</td>
</tr>
<tr>
<td>EP</td>
<td>Embanchment place</td>
<td>PedL</td>
<td>Peduncle Length</td>
</tr>
<tr>
<td>LIT</td>
<td>Leaf Indumentums Type</td>
<td>EPhR</td>
<td>External Phyllary Row</td>
</tr>
<tr>
<td>PBL</td>
<td>Petiole in Basal Leaf</td>
<td>AL</td>
<td>Awns Length</td>
</tr>
<tr>
<td>PCL</td>
<td>Petiole in Cauline Leaf</td>
<td>PhAL</td>
<td>Phylary Appendix Length</td>
</tr>
<tr>
<td>BLT</td>
<td>Basal Leaf Type</td>
<td>NPhAC</td>
<td>Number of Phylary Appendix Cilium</td>
</tr>
<tr>
<td>CLT</td>
<td>Cauline leaf Type</td>
<td>IL</td>
<td>Inflorescence Length</td>
</tr>
<tr>
<td>CLA</td>
<td>Cauline leaf Apex</td>
<td>RL</td>
<td>Radiate floret Length</td>
</tr>
<tr>
<td>PH</td>
<td>Pedicle of Head</td>
<td>AcL</td>
<td>Achns Length</td>
</tr>
<tr>
<td>IPhH</td>
<td>Indumentums in Pedicle of Head</td>
<td>PL</td>
<td>Pappus Length</td>
</tr>
<tr>
<td>ESh</td>
<td>Envelope Shape</td>
<td>PR</td>
<td>Pappus Row</td>
</tr>
<tr>
<td>IPhA</td>
<td>Internal Phyllary Apex</td>
<td>IPhL</td>
<td>Internal Phyllary Length</td>
</tr>
<tr>
<td>IPhT</td>
<td>Internal Phyllary Type</td>
<td>EPhL</td>
<td>External Phyllary Length</td>
</tr>
<tr>
<td>TA</td>
<td>Tooth in base of Awns</td>
<td>MLL</td>
<td>Medial Leaf Length</td>
</tr>
<tr>
<td>LBL</td>
<td>Lacinia in Base of Limbus</td>
<td>MLW</td>
<td>Medial Leaf Width</td>
</tr>
<tr>
<td>IF</td>
<td>Indumentums in Filament</td>
<td>EPhR</td>
<td>External Phyllary Row</td>
</tr>
<tr>
<td>BF</td>
<td>Bulge in base of Filament</td>
<td>IPhR</td>
<td>Internal Phyllary Row</td>
</tr>
<tr>
<td>FC</td>
<td>Flower Color</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AC</td>
<td>Achene Color</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AI</td>
<td>Achene Indumentums</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IPC</td>
<td>Internal Pappus Color</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Anatomy**

This study was conducted on 25 populations of 5 taxa of *Zoegea* including *Z. purpurea*, *Z. crinita* subsp. *crinita*, *Z. crinita* subsp. *baldschuanica*, *Z. crinita* subsp. *glabricaulis*, and Z. Leptaurea subsp. *Mianensis* (Table 1). No specimen of Leptaurea subsp. *mesopotamica* was available. Leaf and stem anatomy, leaf epidermis, achene and pollen morphology were examined in anatomical study. Leaves were fixed in 70% ethanol and stems in alcohol/glycerine (1:1). Cross sections of the middle part of blade and the third internode of the stem were used and double stained by methyl green and Carmine. Appropriate samples were photographed by means of an Olympus BX51 light microscope. The epidermis was prepared using the mixture of H₂O₂ and 5% sodium hydroxyl. The following equation was used to calculate the stomatal index: SI= S/E+ S (SI: Stomatal index; S: guard cells; E: epidermal cells).

**Palynology**

In order to study the pollen grains, flowers were kept in a mixture of absolute acetic acid and ethyl alcohol (96%) 1:1 for 24 hours. The pollen was stained with Carmin and observed by means of an Olympus BX51 light microscope. In order to perform scanning electron microscopy, pollens were coated with a thin layer of gold and observed by means of a SEMTESCAN model VEGA3 Company. About 10-20 pollens were used in measuring polar axis, colpus length, equatorial axis, pore diameter, exin thickness, number and length of spines. Terminology follows Erdtman (1943). Herbarium codes used follows Thiers (2014).

**RESULTS AND DISCUSSION**

**Morphology**

In the dendrogram drawn by Ward’s method (Fig. 1), based on leaf character at 25 level, two clusters were separated including *Z. crinita* subsp. *baldschuanica* and *Z. crinita* subsp. *glabricaulis* in one cluster and the rest taxa in the other. Another notable point is that the *Z. crinita* subsp. *crinita* has more affinity with other species of this genus in comparison with the two other subspecies of *Z. crinita*. Results of CVA analysis based on quantitative and qualitative traits have been shown in Fig. 2 and Fig. 3, respectively. This analysis also determined most important quantitative characters (Table 3). Among them, radiate floret length, inflorescence length, pappus row and number of phyllary appendix ciliun showed the highest correlation in the first function. Medial leaf width and pappus length showed largest absolute correlation with the second discriminant function. In the third function external phyllary row, medial leaf length and herb length showed the largest absolute correlation.
Mahmoodi et al. Morphological and palynological studies in the Zoegea L.

Fig. 1. The dendrogram of cluster analysis by Ward method on the quantitative morphological characters.

Fig. 2. CVA analysis based on main quantitative morphological characters.
In the fourth function external phyllary length, internal phyllary length and phyllary appendix length also showed the largest absolute correlation. Also, CVA analysis determined the most important qualitative characteristics (Fig. 3). The first function did not show any correlated characters. In the second function character of leaf indumentums type showed correlation between species. Indumentums type, flower color, internal pappus color, achene indumentums, indumentums in pedicle of head, internal phyllary type, internal phyllary apex and achene color showed the largest absolute correlation in the third function. In addition, in the fourth function embranchment place, petiole in basal leaf, envelope shape and cauline leaf apex showed absolute correlation (Table 4).

Table 3. The list of quantitative morphologic characters ranked by CVA analysis.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Function</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiate floret length</td>
<td></td>
<td>.725*</td>
<td>.021</td>
<td>.082</td>
<td>-.003</td>
</tr>
<tr>
<td>Inflorescence length</td>
<td></td>
<td>.574*</td>
<td>.034</td>
<td>.310</td>
<td>.117</td>
</tr>
<tr>
<td>Pappus row</td>
<td></td>
<td>.520*</td>
<td>-.078</td>
<td>.115</td>
<td>.224</td>
</tr>
<tr>
<td>Number of phyllary appendix cilium</td>
<td></td>
<td>.404*</td>
<td>-.035</td>
<td>-.075</td>
<td>.376</td>
</tr>
<tr>
<td>Achene length</td>
<td></td>
<td>-.024*</td>
<td>.011</td>
<td>.005</td>
<td>.005</td>
</tr>
<tr>
<td>Pappus length</td>
<td></td>
<td>.216</td>
<td>.540*</td>
<td>.094</td>
<td>.242</td>
</tr>
<tr>
<td>Medial leaf width</td>
<td></td>
<td>.078</td>
<td>.321*</td>
<td>.122</td>
<td>-.140</td>
</tr>
<tr>
<td>External phyllary row</td>
<td></td>
<td>.216</td>
<td>.223</td>
<td>.599*</td>
<td>.008</td>
</tr>
<tr>
<td>Medial leaf length</td>
<td></td>
<td>-.099</td>
<td>-.112</td>
<td>.475*</td>
<td>-.012</td>
</tr>
<tr>
<td>Herb length</td>
<td></td>
<td>-.074</td>
<td>.118</td>
<td>.391*</td>
<td>-.203</td>
</tr>
<tr>
<td>External phyllary length</td>
<td></td>
<td>-.087</td>
<td>.220</td>
<td>.422</td>
<td>.592*</td>
</tr>
<tr>
<td>Internal phyllary length</td>
<td></td>
<td>.354</td>
<td>.006</td>
<td>.132</td>
<td>.512*</td>
</tr>
<tr>
<td>Internal phyllary rowa</td>
<td></td>
<td>.354</td>
<td>.334</td>
<td>.315</td>
<td>.415*</td>
</tr>
<tr>
<td>Phyllary appendix length</td>
<td></td>
<td>.354</td>
<td>.334</td>
<td>.315</td>
<td>.415*</td>
</tr>
</tbody>
</table>

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions
Variables ordered by absolute size of correlation within function.

* Largest absolute correlation between each variable and any discriminant function
a. This variable not used in the analysis.
Mahmoodi et al. Morphological and palynological studies in the Zoegea L.

Table 4. The list of qualitative morphologic characters ranked by CVA analysis.

<table>
<thead>
<tr>
<th>Structure Matrix</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf indumentums type</td>
<td>-.067</td>
<td>.561*</td>
<td>.291</td>
<td>.080</td>
</tr>
<tr>
<td>Indumentums type</td>
<td>.025</td>
<td>-.256</td>
<td>.731*</td>
<td>.203</td>
</tr>
<tr>
<td>Flower color</td>
<td>.188</td>
<td>-.075</td>
<td>-.435*</td>
<td>-.032</td>
</tr>
<tr>
<td>Internal pappus color</td>
<td>-.175</td>
<td>.041</td>
<td>.366*</td>
<td>-.255</td>
</tr>
<tr>
<td>Achene indumentums</td>
<td>.302</td>
<td>-.076</td>
<td>-.349*</td>
<td>-.026</td>
</tr>
<tr>
<td>Indumentums in pedicle of head</td>
<td>.040</td>
<td>.143</td>
<td>.343*</td>
<td>-.021</td>
</tr>
<tr>
<td>Internal phyllary type</td>
<td>.117</td>
<td>-.026</td>
<td>-.323*</td>
<td>.299</td>
</tr>
<tr>
<td>Internal phyllary apex</td>
<td>.178</td>
<td>-.045</td>
<td>-.206*</td>
<td>-.016</td>
</tr>
<tr>
<td>Achene color</td>
<td>-.001</td>
<td>-.014</td>
<td>-.168*</td>
<td>.076</td>
</tr>
<tr>
<td>Basal leaf type</td>
<td>-.030</td>
<td>-.006</td>
<td>-.127*</td>
<td>.075</td>
</tr>
<tr>
<td>Embranchment place</td>
<td>.018</td>
<td>-.051</td>
<td>-.171</td>
<td>.551*</td>
</tr>
<tr>
<td>Petiole in basal leaf</td>
<td>.035</td>
<td>-.006</td>
<td>-.201</td>
<td>.262*</td>
</tr>
<tr>
<td>Envelope shape</td>
<td>-.167</td>
<td>.061</td>
<td>.211</td>
<td>.219*</td>
</tr>
<tr>
<td>Cauline leaf apex</td>
<td>-.041</td>
<td>-.013</td>
<td>-.073</td>
<td>-.105*</td>
</tr>
</tbody>
</table>

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions

<table>
<thead>
<tr>
<th>Variables ordered by absolute size of correlation within function.</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Largest absolute correlation between each variable and any discriminant function</td>
</tr>
</tbody>
</table>

Anatomy
Anatomical results of the leaf showed that qualitative characters including cuticle, secretory, transport tissue, number of layers of ladder parenchyma, type of ladder parenchyma, sclerenchyma, collenchymas, central vascular bundle sheath, fiber and trichome were similar in all Zoegea species. Also, stem characters couldnot show the separation between taxa. Clustering resulting from anatomy was not the same as morphological analysis and showed the close relationship between the Z. crinita subsp. crinita and Z. leptaurea subsp. mianensis in comparison with the two other subspecies of Z. crinita (Fig. 7). Stoma type in all taxa was Anomocytic, however, at the ventral surface of Z. crinita subsp. baldschuanica and Z. crinita subsp. Glabricaulis the anisocytic type and paracytic type were also observed, respectively (Figs. 4, 5 & 6 and Tables 5 & 6).

Table 5. Results obtained from qualitative and quantitative measurements of leaf (μm).

<table>
<thead>
<tr>
<th>Species</th>
<th>Cuticle</th>
<th>Secretory</th>
<th>Transport Tissue</th>
<th>number of layers of ladder parenchyma</th>
<th>Type of ladder parenchyma</th>
<th>Sclerenchyma</th>
<th>Collenchyma</th>
<th>Central vascular bundle sheath</th>
<th>Fiber</th>
<th>Trichome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z. crinita subsp. crinita</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2-3</td>
<td>Bilateral</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Z. crinita subsp. baldschuanica</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>Bilateral</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Z. crinita subsp. glabricaulis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>Bilateral</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Z. Leptaurea L. subsp. mianensis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>Bilateral</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Z. purpurea</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>Bilateral</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Fig. 4. Cross section of leaf in species studied: A, B: Zoegealeptaurea subsp. Mianensis; C, D: Z. crinita subsp. crinita; E, F: Z. crinita subsp. Baldschuanica; G, H: Z. crinita subsp. glabricaulis (I, J, K) Z. purpurea. (E) Epidermis, (Ph) phloem, (Xy) Xylem, (Parenchyma ladder), (Se) Secretory, (VBSh) Vascular Bundle Sheath, (TT) Transport Tissue, (Sc) Sclerenchyma, (Co) Collenchyma, (F) Fiber.

Fig. 5. Upper epidermis of leaf in species studied: A: Zoegeacrinita subsp. baldschuanica; B: Z. crinita subsp. crinita; C: Z. crinita subsp. glabricaulis; D: Z. Leptaurea subsp. mianensis; E: Z. purpurea.
Fig. 6. Lower epidermis of leaf in some studied species: A: Zoegeacrinita subsp. baldschuanica; B: Z.crinita subsp. glabricaulis; C: Z.leptaurea subsp. mianensis.

Table 6. Results of the study on features of leaf epidermis, stoma and trichome.

<table>
<thead>
<tr>
<th>Species</th>
<th>Type of Epidermis</th>
<th>Type of epidermal cell walls</th>
<th>Type of stoma</th>
<th>Type of trichome</th>
<th>Stomatal Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z.crinita subsp. crinita</td>
<td>Dorsal</td>
<td>Polygonal</td>
<td>Anomocytic</td>
<td>Simple</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Ventral</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Z.crinita subsp. baldschuanica</td>
<td>Dorsal</td>
<td>Polygonal</td>
<td>Anomocytic</td>
<td>Simple</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Ventral</td>
<td>Polygonal - Flexuose</td>
<td>Anomocytic + Anisocytic</td>
<td>Simple</td>
<td>10</td>
</tr>
<tr>
<td>Z.crinita subsp. glabricaulis</td>
<td>Dorsal</td>
<td>Polygonal</td>
<td>Anomocytic</td>
<td>Simple</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Ventral</td>
<td>Polygonal - Flexuose</td>
<td>Anomocytic + Paracytic</td>
<td>Simple</td>
<td>11</td>
</tr>
<tr>
<td>Z.leptaurea subsp. mianensis</td>
<td>Dorsal</td>
<td>Polygonal</td>
<td>Anomocytic</td>
<td>Simple</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Ventral</td>
<td>Polygonal - Flexuose</td>
<td>Anomocytic</td>
<td>Simple</td>
<td>8</td>
</tr>
<tr>
<td>Z.purpurea</td>
<td>Dorsal</td>
<td>Polygonal</td>
<td>Anomocytic</td>
<td>Simple</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Ventral</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 7. The results of the cross section of cypsela measured by Digimizer software (Measurements are in (μm)).

<table>
<thead>
<tr>
<th>Species</th>
<th>Cuticle</th>
<th>Secretory channel</th>
<th>Length of Epicarp</th>
<th>Length of Mesocarp</th>
<th>Length of Testa</th>
<th>Length of middle Testa</th>
<th>Length of inner Endosperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z. crinita subsp. crinita</td>
<td>+</td>
<td>+</td>
<td>12.58</td>
<td>81.49</td>
<td>45.79</td>
<td>67.34</td>
<td>15.89</td>
</tr>
<tr>
<td>Z. crinita subsp. baldschuanica</td>
<td>+</td>
<td>-</td>
<td>11.06</td>
<td>0</td>
<td>76.83</td>
<td>116.20</td>
<td>13.69</td>
</tr>
<tr>
<td>Z. crinita subsp. glabricaulis</td>
<td>-</td>
<td>+</td>
<td>15.75</td>
<td>120.40</td>
<td>65.07</td>
<td>47.61</td>
<td>18.10</td>
</tr>
<tr>
<td>Z.leptaurea subsp. mianensis</td>
<td>-</td>
<td>+</td>
<td>11.29</td>
<td>55.08</td>
<td>27.07</td>
<td>49.93</td>
<td>7.32</td>
</tr>
<tr>
<td>Z.purpurea</td>
<td>-+</td>
<td>-+</td>
<td>6.65</td>
<td>30.28</td>
<td>23.09</td>
<td>39.72</td>
<td>5.29</td>
</tr>
</tbody>
</table>
Anatomical results of cypsela showed that the testa was made of three layers: epidermis layer, collenchyma layers under the epidermis, and sclerenchyma layers in the middle. Inner layers made of parenchyma and one vascular bundle could be found in each of them (Fig. 8). Analysis of anatomical characters measured in cypsela provided useful information. Chart of PCA (principal component analysis) obtained from quantitative characters of cypsela showed in Fig. 9. Traits of the first factor (i.e., the thickness of the epicarp layers, testa and the inner endosperm) with high correlation (>0.8) with a share of 77% of the total variance could separate Z. purpurea isolated from other species in the vertical axis. Characters of the second factor (i.e., the thickness of the middle testa layer and testa) with a correlation coefficient (>0.3) with a share of 29% of the total variance separated the subspecies of Z. crinita from other species in the horizontal axis. In all taxa prismatic crystals were found in the testa layer and the vascular tissue in the middle testa (Table 7 and Fig. 9).

**Palynology**

Pollen in all specimens of Zoegea which were studied was tricolporate and three pore were seen in polar view as bulge. Exine structure was covered with spines in all taxa that were somewhat different in length, but covered the entire pollen surface uniformly (Table 8 and Fig. 10). The longest length of the polar axis was observed in Z. crinita subsp. baldschuanica and the shortest length was observed in Z. purpurea. Also, the longest equatorial axis length was observed in Z. crinita subsp. baldschuanica and the shortest axis of tropical was observed in Z. leptaurea subsp. mianensis. The length of spine of the pollen in Z. leptaurea subsp. mianensis was more than the other taxa.

Morphological characters showed that Z. purpurea was well-separated from other species. Results of CVA analysis based on quantitative characters showed that Z. crinita subsp. baldschuanicawasa completely distinct taxon that could easily be differentiated from Z. crinita. But in this analysis based on qualitative characters some specimens of Z. crinita subsp. baldschuanica occurred near other members of Z. crinita.

Also, morphological characters (i.e., leaf indumentums type, indumentums type, flower color, embranchment place, radiate floret length, pappus length, external phyllary length, etc) (Tables 3 & 4) showed that Z. crinita subsp. glabricaulis was separate from Z. crinita. Results of cypsela anatomical studies confirmed morphological results. Morphological characters could not separate Z. crinita and Z. leptaurea subsp. mianensis from each other. However, the cluster analysis by ward’s method based on quantitative morphological characters could separate Z. crinita and Z. leptaurea subsp. mianensis.
Fig. 9. PCA of the first and second factors based on Morphological quantitative cypselas features.

Since the morphological characters used in identification keys of taxonomic literatures are not suitable to distinguish these taxa. Therefore, we restablished the following taxa on the basis of the result of this study.

**Zoegea baldschuanica** C.Winkl.
Syn.: **Zoegea crinita** Boiss. subsp. *baldschuanica* (C.Winkl.) Rech.f.

**Zoegea glabricaulis** Czerep.
Syn.: **Zoegea crinita** Boiss. subsp. *glabricaulis* (Czerep.) Rech.f.

**Table 8.** Results obtained from evaluation of quantitative and qualitative features of pollen grains (size= µm).

<table>
<thead>
<tr>
<th>Species</th>
<th>Polar axis Length (P)</th>
<th>Equatorial axis length (E)</th>
<th>P/E</th>
<th>For m</th>
<th>Length of spine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minim um</td>
<td>Maxim um</td>
<td>Average</td>
<td>Minim um</td>
<td>Maxim um</td>
</tr>
<tr>
<td><em>Z. crinita</em> subsp. crinita</td>
<td>30.79</td>
<td>31.04</td>
<td>30.92</td>
<td>30.12</td>
<td>35.00</td>
</tr>
<tr>
<td><em>Z. crinita</em> subsp. <em>baldschuanica</em></td>
<td>31.61</td>
<td>36.75</td>
<td>34.18</td>
<td>31.42</td>
<td>37.62</td>
</tr>
<tr>
<td><em>Z. crinita</em> subsp. <em>glabricaulis</em></td>
<td>26.82</td>
<td>28.75</td>
<td>27.78</td>
<td>25.89</td>
<td>28.88</td>
</tr>
<tr>
<td><em>Z. purpurea</em></td>
<td>22.93</td>
<td>24.70</td>
<td>23.81</td>
<td>29.00</td>
<td>29.37</td>
</tr>
</tbody>
</table>

P: Polar axis, E: Equatorial axis, Cl: Colpus Length, OS: Oblate Spheroidal, SO: Suboblate
CONCLUSION

We concluded that Zoegea baldschuanica and Z. glabricaulis were independent species. Also, it was shown that anatomical characters such as stomatal index, existence of trichome, number of ladder parenchyma layers, thickness of epicarp layers, testa layers and inner endosperm and existence of secretory channel were diagnostic characters in distinguishing the species of Zoegea.

ACKNOWLEDGEMENT

The authors are grateful to the curators of the herbaria TARI, TUH and the herbarium of Shiraz University (Shiraz, Iran).

REFERENCES


How to cite this article: